UNIVERSITY of LIFE SCIENCES in Lublin
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF BIOCHEMISTRY
20-033 Lublin, Akademicka 12
Phone +48 81 445 66 08
www.biochwet.up.lublin.pl

Enzyme kinetics

Task 1. Study of the effect of enzyme concentration and reaction time on the rate of enzymatic reaction

The aim of the task is to observe the influence of enzyme concentration and time of enzyme action on the velocity of sucrose hydrolysis by invertase.

The principle of determination

Sucrase (invertase) belongs to the hydrolases that cleave the glycosidic bond in sucrose, breaking it down into glucose and fructose. This process is called inversion, hence the second name of the enzyme is invertase. The optimal pH of this enzyme is between 4-7; at pH=10 it is completely inactive.

Invertase activity may be measured by the method for the detection of reducing sugars, because the concentration of simple sugars (glucose and fructose) increases as the hydrolysis progresses.

To determine the amount of copper oxide I formed during the oxidation of sugars, the reaction with a phosphomolybdenum reagent is conducted. The reaction leads to the reduction of phosphomolybdenum reagent to molybdenum blue. The intensity of the blue color is proportional to the amount of reducing sugar.

Procedure

Add 1 cm^3 of copper reagent, 0.7 cm^3 of distilled water and 0.1 cm^3 of 0.05 mol/dm^3 NaOH, respectively, to 10 numbered test tubes (1,2,3… etc.)

Add 0.1 cm^3 of undiluted enzyme and 0.1 cm^3 of distilled water to the test tube 1.

Add 0.1 cm^3 of 2x diluted enzyme and 0.1 cm^3 of distilled water to the test tube 6.

This set is used to visualize the effects of the enzyme during the reaction. Test tube 1 is a control for the tubes: 2,3,4,5; test tube 6 - a control for the tubes with diluted enzyme: 7,8,9,10.

Prepare an incubation system consisting of 1 cm^3 of 0.8 mol/dm^3 sucrose preheated to 37°C and 1 cm^3 of the enzyme solution in the test tube A. Immediately put the tube into a water bath (37°C) and note time "0" - the moment of mixing the substrate

with the enzyme. It should be remembered that the substrate concentration after adding the enzyme is 2x lower.

After 5, 10, 20 and 30 mins of incubation, take 0.2 cm^3 of the incubate from tube A into previously prepared tubes with the copper reagent:

- after 5 mins to the test tube 2,
- after 10 mins to the test tube 3,
- after 20 mins to the test tube 4,
- after 30 mins to the test tube 5.

Prepare an incubation system consisting of 1 cm^3 of 0.8 mol/ dm^3 sucrose preheated to 37°C, 0.5 cm^3 of the enzyme solution (2x diluted) and 0.5 cm^3 of distilled water in the test tube B. Immediately put the tube into a water bath (37°C) and note time "0".

After 5, 10, 20 and 30 mins of incubation, take 0.2 cm^3 of the incubate from tube B into previously prepared tubes with the copper reagent:

- after 5 mins to the test tube 7,
- after 10 mins to the test tube 8,
- after 20 mins to the test tube 9,
- after 30 mins to the test tube 10.

At the end of incubation, all test tubes (10) heat for 8 minutes in a boiling water bath, cool, and then add 1 ml of phosphomolybdenum reagent to all tubes. Mix thoroughly!!! Next, dilute the tubes depending on the intensity of the color, and measure the absorbance of the samples in the spectrocolorimeter at 610 nm: test tubes 2, 3, 4, 5 against tube 1 and test tubes 7, 8, 9, 10 against tube 6.

Draw the standard curve, plotting the absorbance on the Y axis, and the time of enzyme action and on the X axis (2 curves for two different concentration of the enzyme in 1 chart).